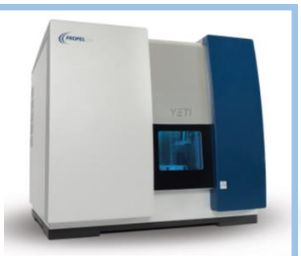
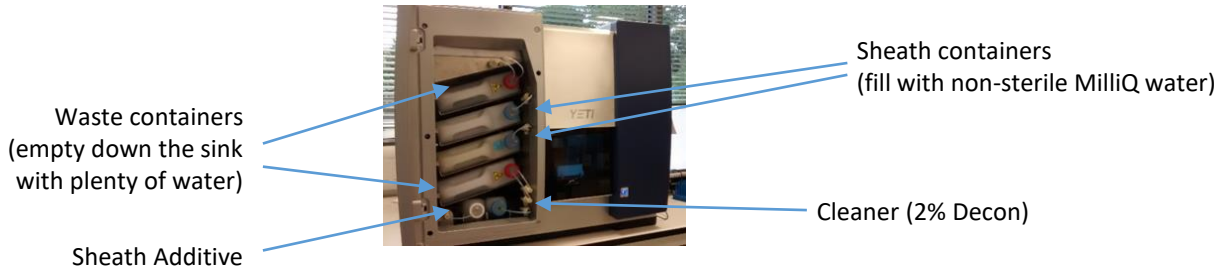
Yeti User Guide



Before you begin:

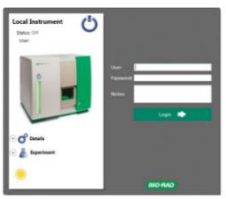
- Check the fluidics levels. Empty the waste down the sink (containers with red lids). Add a small amount of Chemgene to the bottom of the empty waste container before placing back on the machine. Fill the sheath containers (blue lids) with distilled water to the fill line.

- Check the levels of cleaner (2% Decon) and sheath additive. Replace/top-up if necessary.



Start-up:

- Double click the Everest software icon.



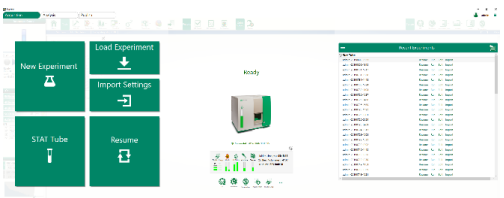
- The log-in screen should appear. Log in using the username ‘admin’ and the password ‘admin’.

- Click the Startup icon to begin start-up.

- The instrument status should change to Ready.

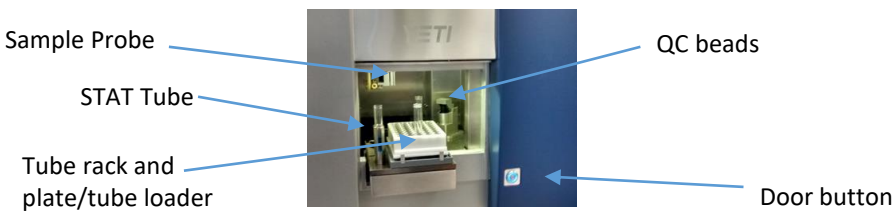
- The Everest home window appears.

- Select New Experiment.



QC:

- Run the automated QC process if required. QC needs to run once a week. Ensure that the QC bead bottle is present to the right side of the sample chamber.



- In the toolbar click the QC down arrow to monitor the progress of the QC. The QC measure the CV of the beads in each channel. The YETI EYE monitors the filter configuration.

New Experiment:

- From the Home window choose New Experiment.
- Note that you can also load/edit a previous experiment from the list on the right hand side of the Home window.
- Enter an experiment name and click next. This takes you to the fluorophore selection screen.
- Select each fluorophore you want to add to the experiment either by double-clicking on the name or entering the name at the top and then double clicking. Select Enable All if you want to keep all channels open.
- Add your axis labels here. Double-click on the fluorophore name in the assigned channel and write in your label. Label the filter, laser, fluorophore, and target in this field.

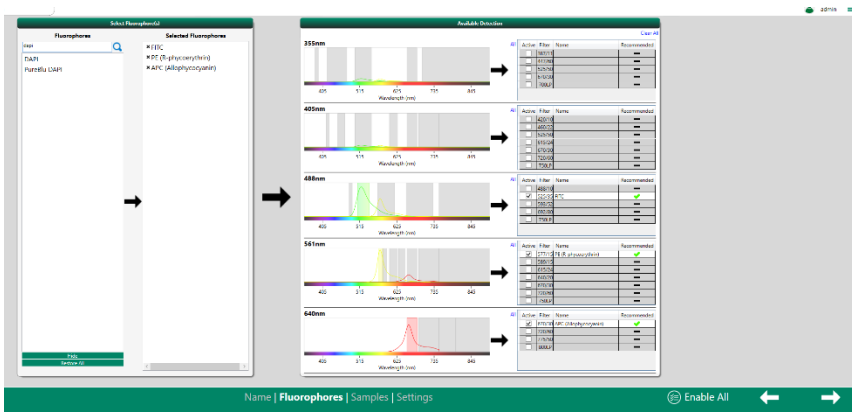
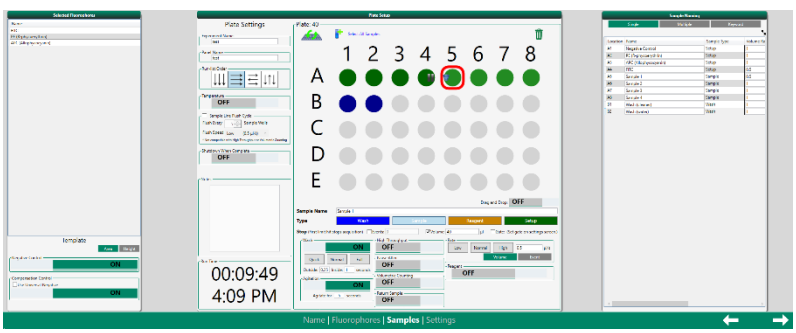


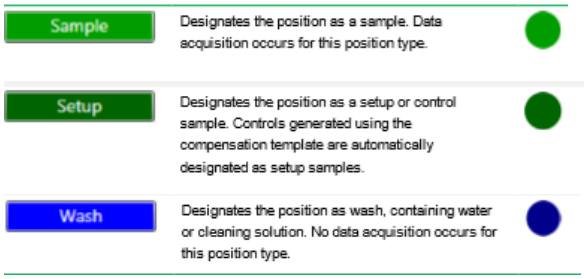
Plate setup panel:

- Select a media type.

- This takes you to the plate setup screen. Here you can configure the tube rack or plate according to the requirements of your experiment.

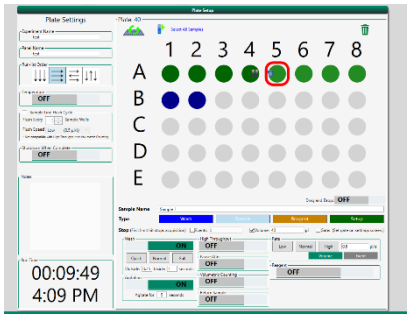


- Make sure to use setup wells for controls (dark green), wash wells (blue) for cleaning, and sample wells (light green) for your experimental samples. Include regular wash wells/tubes to prevent clogging.

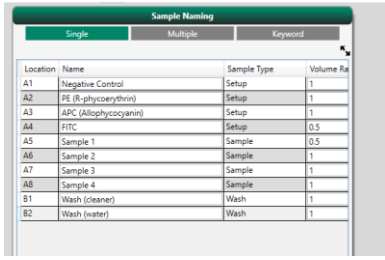


- Ensure that settings are applied to the appropriate wells by making sure all wells are highlighted before you select the settings you require for your experiment. Use drag and drop to move wells around according to your plate layout. Settings such as temperature control, run-list order, acquisition limits, wash setting, agitation etc. are all available on this screen.

- Use the drag and drop option to arrange the tubes/wells to the order that they are in your tube rack or plate.



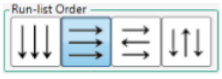
- Use the sample naming panel to type your sample names. The double ended arrow icon can be used to expand the run-list to check the order of samples and the settings applied to each sample. Use this before proceeding.



- Other settings that can be applied are explained below.

Run list order:

- In the plate setup screen you can also select the run-list order using the icon shown below.

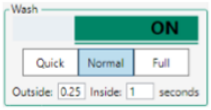


Temperature control settings:

- Temperature control settings for the sample stage are also available on this screen. Please do not leave the machine running at below room temperature for longer than your experiment requires.

Wash settings:

- Please select normal wash settings. This washes the inside and outside of the probe and is selected by default. Include a wash step for every well/tube.



Sample agitation:

- You can select 'Agitation' for any position by toggling the agitation ON after selecting the sample/well. A symbol like this will appear: 

'Pause After' feature:

- When 'Pause After' is activated, the acquisition will stop after that well. This allows compensation to be calculated before continuing or other inputs to be made. It is most useful to use this after your last compensation control to allow you to analyse the controls and apply compensation to your experiment. This symbol will appear on a well/tube when 'Pause After' is selected: 

Setting the flow rate:

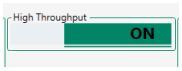
- Flow rates: Low flow rate is 0.5µl/s (this is equivalent to running on the medium setting of the Fortessa analysers). Select the flow rate that is appropriate for your experiment.

'Return Sample' feature:

- 'Return Sample': toggle to ON if you want to conserve sample. After the acquisition limit has been reached the sample pump runs backwards to return any unused sample to the tube/well.

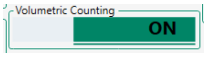
High throughput versus standard throughput modes:

- In standard mode only one sample is in the sample line at a time. This continues until the event limit, volume limit, or gate limit is reached.
- In high throughput mode samples are aspirated continuously and each sample is separated by a series of air and water boundaries. Only volume limits can be applied in this mode.
- Toggle the High Throughput button to ON to use in high throughput mode.



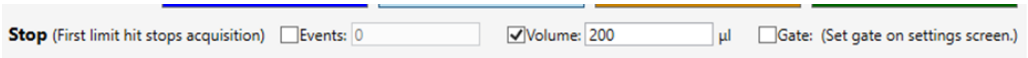
Volumetric counting:

- This allows the number of particles in a specified volume to be counted.
- The volume stop limit for this is 10µl. After a boost, the software runs 5µl of sample to stabilise the flow before acquisition begins to acquire the 10µl.
- 'Return Sample' is on by default.
- Toggle the 'Volumetric Counting' to ON to use this feature.



Setting acquisition limits (number of events to record):

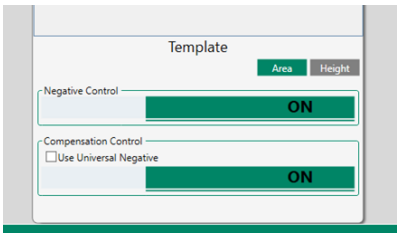
- Use this selection box to pick an acquisition limit:



- If multiple limits are set, acquisition will stop at whichever is reached first.
- In the plate setup screen select the checkbox for the limit you want.
    - Event limit – stops when the total event count is reached.
    - Volume limit – stops when volume is reached. Volume limits are used to calculate plate run time.
    - Gate limit - sets the volume limit to max for the media type selected. Configure the actual gate limit (e.g.10,000 events of region R1) properly in the settings screen. Type in the maximum volume in each tube/well.
- If you want to set a gate limit tick the gate limit box on the plate set-up screen but do not set a number. Specify the actual gate limit on the settings screen of the experiment builder.

Compensation:

- Activate the compensation template by moving the compensation controls toggle to ON.



- Plate positions are automatically filled, with each control having a designated workspace.
- Check the run-list for the order of the controls.
- To label positions manually, highlight the well and then type a name in the sample name box (located below the plate map). Press enter to move to the next row.
- To include a negative control tube or well in the experiment select the negative control button and move to the ON position.
- To use this negative control as a negative for all colours tick the box that says ‘Use Universal Negative’. If this is left unticked the negative population from each single colour will be used instead.
- Ensure that the pulse parameter has Area selected (this should be green).

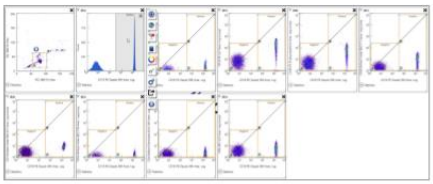
- Select the last single colour control and add a Pause After to this sample by highlighting the Pause After function in green. This will stop acquisition after this tube so that compensation can be calculated and applied to the experiment before the fully stained samples are run.

- To select voltages run the fully stained sample in the negative control tube workspace (in Setup mode).

- Make sure that voltages are set so that at least 90% of the negatives are on scale and also ensure that all positive populations are also on scale.

- Run all the compensation controls in Acquisition mode. Do not use eBioscience beads for controls. Use BD beads or preferably cells.

- If you are running a combination of cells and beads for your single colour controls then either find a FSC and SSC voltage that works to put both on scale or run the cell controls at their optimal scatter voltages and then change the FSC and SSC voltages to run the beads at their optimal scatter voltages.



- Click on analyse to open the analysis workspace for compensation.

- To calculate compensation click the down arrow next to the compensation button and then click calculate.

- Open the comp matrix to check the values and review the compensated control plots.

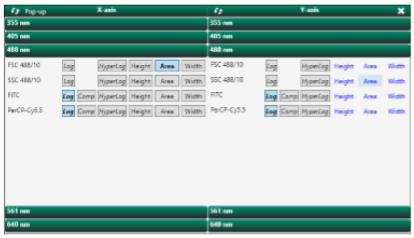
- To apply this compensation to the experiment and continue running samples click Send To Instrument.

- In the Acquisition tab proceed by selecting the sample you want to start with and then push the Run button.

Experiment configuration panel (plots and gating):

- Select a sample and create plots and regions for analysis. You can adjust any thresholds or PMT voltages at this stage but it is often easier to do this later.
- To select plots click on the histogram or dot plot icon and then select the parameter for each axis.

- Select the parameter for each axis. Note that you can display uncompensated and compensated data in the same workspace. Select the comp parameter for plot axes to make sure that the compensation matrix is applied to the data.



- To select a region hover over the plot and select the add region tool . This can be a rectangle region or a polygon region. Click around your population to create a gate. To rename a gate double click and then rename it.

- To create a gating hierarchy hover over the next plot and select the apply filter icon. Select the region you want to apply to the plot.

- The gating hierarchy will appear at the top of each plot. Note that regions with ~ prefix are 'NOT' gates so ~R1 will be everything outside of region R1.

- To apply a gate limit for samples, find the gate limit symbol on the toolbar (hand symbol). Select a region and then type in a limit for recording. Click 'Sample' to apply this to all experimental samples.

- Once your experiment is setup then click Apply.

Running samples:

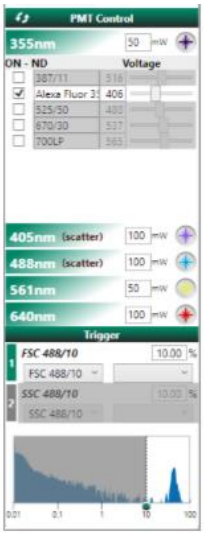
- The configured workspace will appear in setup mode. The instrument control panel displays setup options.

- To run samples in setup mode click the run sample icon. Note that samples can also be recorded in setup mode using the record button.

- While acquiring you can adjust the PMT voltages to place populations on scale. To do this either use the slider bars next to the detector name or you can scroll using the mouse wheel or simply type in a voltage value.

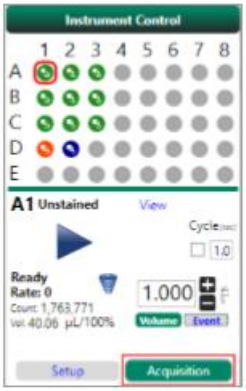
- When adjusting voltages it is a good idea to reduce down the flow rate to 0.5µl/s or less so you don't use so much of your sample.

- Selecting the cycle checkbox will cycle your data so that it refreshes as you change settings.



- Once you are happy with your settings click on Acquisition to move into Acquisition mode.

- Click the run list Start icon to begin sampling. The samples should be acquired and recorded until the end of the run-list is reached.
- To stop acquiring manually click stop or to skip a well click on the arrow.



Exporting fcs files:

- You can export for a single position or all positions. Click Export Data in the tools section of the toolbar.

- This brings up an export dialog box. Positions that will export are selected by a green tick. Select fcs files. Make sure area height and width is ticked for FSC and SSC. Area should be ticked for Fluorescence parameters.

- Make sure that the tick box next to convert for third party analysis is unticked unless you are looking at data in Cytobank.

- Export your files to the Users FCS Data folder on the desktop.

- Make your own folder within this folder and save all your experiments in this folder.

- This folder will be backed up regularly by the flow facility.

Pausing the system:

- Pausing the system can conserve sheath fluid. Please use this feature if you are leaving the machine on for significant lengths of time. Use the pause and resume icons (located under instrument tools in the toolbar) to stop and restart the fluidics.

Cleaning:

- In the home window click Stat Tube.

- Place a tube of bleach (FACS Clean) in the tube holder.
- Select Run. **Run bleach for at least five minutes.**

- Then place a tube of detergent (Coulter Clenz) in the tube holder.
- Select Run**. Run detergent for at least five minutes.**

- Place a tube of water in the tube holder.
- Select Run. **Run water for at least five minutes.**

- **This must be done after every experiment run with no exceptions.**

- If you have been using CL2 samples (which should be fixed before bringing to the facility) then please wipe down the keyboard, mouse and any buttons with **Chemgene wipes.**

Longer cleans and in case of blockage:

- The software includes an option to initiate a sample line and probe clean cycle.
- This runs the on-board cleaner through the system. To run this select the clean icon. Run QC after to check that the clean has worked. 

- Unclogging the sample line and probe. In the system part of the toolbar select the Unclog option. Click the down arrow to follow the process. Run QC after to check the unclog has worked. 

Shutdown:

- In the toolbar click the shutdown icon. 

- The system will turn off all the lasers, rinse the system with cleaner, depressurise the fluidics and put the instrument in a sleep state until start-up is run again.